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obstructive pulmonary disease (COPD), a urological disorder such as neuropathy, incontinence or interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR, preferably hVR1 or hVR3, activity in a human patient which comprises administering to said patient an effective amount of a compound identified by the method referred to above. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) and urological disorders including diabetic neuropathy, incontinence and interstitial cystitis and inflammatory disorders.

According to another aspect of the invention there is provided a method of producing an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, under conditions suitable for obtaining expression of the hVR protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof.

Brief Description of the figures

Figure 1 is an alignment of hVR1 *in silico* derived clusters with rat VR1.

Figure 2 displays the human VR1 nucleotide sequence including the 5'UTR (nt – 773 to nt 0), coding region (nt 1 to 2517) and 3'UTR (nt 2518 to nt 3560) (SEQ ID NO:1).

Figure 3 illustrates the nucleotide and encoded amino acid sequence of the human VR1sequence (SEQ ID NO: 1 and SEQ ID NO:2).

Figure 4 depicts the amino acid sequence (SEQ ID NO: 2) of the hVR1 gene, the shading denotes predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed). The predicted phosphorylation sites are underlined.

Figure 5 is a comparison of the amino acid sequences of the rat (rVR1) (SEQ ID NO:3) and human (hVR1) vanilloid receptors (SEQ ID NO:2).

Figure 6 illustrates constructs pBluescriptSK(+) (A) and pCIN5-new (B) with the full length hVR1 gene cloned via NotI and EcoRI restriction sites.

Figure 7 shows a Slot Blot hybridisation with hVR1 probe with positive labelling of both rat and human DRG mRNA.

Figure 8 displays a Western blot probed with anti-VR1 antibodies with the arrow indicating the VR1 specific protein.

Figure 9 shows localisation of VR1 in rat DRG tissue sections, the arrow points to VR1 expressing small diameter (<25μn) neurone cell bodies.

Figure 10 depicts the *in situ* localisation of VR1 in human DRG sections (A) and human skin (B).

Figure 11 illustrates the functional response to capsaicin and blockade by capsazepine (CPZ) (A) with the current voltage relationship plotted in (B) on human VR-1 channels, transiently expressed in HEK293T cells.

Figure 12 shows capsaicin-induced desensitisation of human VR-1 channels in the presence of 2mM external calcium (A), maximum current (65mV) against time (B) and current voltage relationship in the absense of Ca^{2+} (C).

Figure 13 shows the influx of calcium into transiently transfected HEK293T cells over a time course in the presence of agonist capsaicin, anandamide and resiniferatoxin in the absence (A, B, D and F) or presence (C, E, G) of the antagonist, capsezipine.

Figure 14 illustrates a graphical presentation the results shown in figure 13 examining the response of hVR1 transfected HEK293T cells over time before and after exposure to agonists: capsaicin, anandamide and resiniferatoxin in the absence (A, B, D and F) or presence (C, E, G) of the antagonist, capsezipine.

Figure 15 displays the proposed assay strategy to carry out drug screening.

Figure 16 displays an alignment of *in silico* derived hVR3 specific clusters with rat VR1.

Figure 17 depicts the hVR3 nucleotide sequence including the 5' UTR (nt –686 to nt 0) Coding region (nt1 to nt 2889), 3'UTR (nt 2890 to nt 3418) (SEQ ID NO:4).

Figure 18 shows the nucleotide and amino acid sequence of hVR3 (SEQ ID NO:4 and SEQ ID NO:5).

Figure 19 is of the amino acid sequence of hVR3 (SEQ ID NO:5), the shading denotes predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed).

Figure 20 displays constructs pBluescriptSK(+) (A) and pCDNA3.1 (+) (B) with the full length hVR3 gene cloned via NotI and XhoI restriction sites.

Figure 21 illustrates a multiple comparison of the amino acid sequences of the rat VR1(SEQ ID NO:3) and the human vanilloid receptors: hVR1, hVRL-1 and hVR3 (SEQ ID NO:2), (SEQ ID NO:6) and (SEQ ID NO:5), respectively.

Figure 22 Northern Blot hybridisation with hVR3 probe with strong signals detected in trachea (A), prostate (B), placenta, kidney and pancreas (C).

Detailed Description of the Invention

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

As referred to above, the present invention relates to isolated human vanilloid receptor (hVR) proteins, and in particular to the human vanilloid receptors which will be termed respectively human vanilloid receptors 1 and 3 (hVR1, and hVR3), sequence information for which is provided in figures 2 (hVR1) and 17 (hVR3). In the context of this invention the term "isolated" is intended to convey that the receptor protein is not in its native state, insofar as it has been purified at least to some extent or has been synthetically produced, for example by recombinant methods. The term "isolated" therefore includes the possibility of the receptor protein being in combination with other biological or non-biological material, such as cells, suspensions of cells or cell fragments, proteins, peptides, organic or inorganic solvents, or other materials where appropriate, but excludes the situation where the receptor protein is in a state as found in nature.

Routine methods, as further explained in the subsequent experimental section, can be employed to purify and/or synthesise the receptor proteins according to the invention. Such methods are well understood by persons skilled in the art, and include techniques such as those disclosed in Sambrook, J. *et al.* (28), the disclosure of which is included herein in its entirety by way of reference.

By the term "variant" what is meant throughout the specification and claims is that other peptides or proteins which retain the same essential character of the human vanilloid receptor proteins for which sequence information is provided, are also intended to be included within the scope of the invention. For example,

The compounds may be administered via enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intraarterial, intramuscular, intraperitoneal, topical or other appropriate administration routes.

The present invention will be further explained, by way of examples, in the appended experimental section. Reference examples are provided.

Experimental details

Reference Example A: Identification of related human ESTs (Expressed Sequence Tags) (19) to the rat VR1 sequence by *in silico* analysis

The full-length rat VR1 amino acid sequence (15) was used as a query sequence using the tBlastn (20) alignment program to identify related human genes in the dbEST (21) and Incyte (Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, California 94304, USA) databases. Several human ESTs were identified and those with similarities greater than 50% selected for further analysis. One of these ESTs was T12251 previously shown to have 68% amino-acid identity and 84% similarity over a region of 70 amino acids (15). Full-length cloning and functional characterisation of the gene represented by this cluster has been completed (30). This gene was denoted hVRL-1 and encoded a protein of 764 amino acid protein (SEQ ID NO:6) that was 48 % identical to the rat VR1 protein. All human ESTs from both databases were clustered to identify overlapping identical ESTs belonging to the same transcript. The GCG package (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin) and a program developed in house termed ESTBlast (22) were used to build up these clusters. In total, forty-three ESTs derived from different tissue sources and both EST databases were clustered into ten groups, one of these clusters represented hVRL-1. The remaining nine clusters have been named hVRa, hVRb, hVRc, hVRd, hVRe, hVRf, hVRg, hVRh and hVRi. For each EST the tissue source was assigned according to the annotations in the dbEST and Incyte databases. Since no obvious starting codon was present and the cluster sequences were shorter than the rat VR1 transcript none of these clusters were likely to represent a full-length vanilloid receptor transcript. Finally hVRg, hVRh and hVRi collapsed into a single contig. Sequence analysis has shown that

Refer nce Exampl B2: Sequencing of clon s

All DNA sequences were determined by automated DNA sequencing based on the dideoxy chain-termination method using the ABI 373A / 377 sequencers (Applied Biosystems). Sequence-specific primers were used with the 'Big-Dye' Terminator Cycle Sequencing kit (Applied Biosystems). The nucleotide sequence was analysed using programs from the University of Wisconsin Genetics Computer Group package.

More specifically when sequencing an EST clone, the following protocol was adopted. The EST1 clone was grown using standard procedures and DNA was isolated using Qiagen columns. SP6 (5' ATTTAGGTGACACTATAG) (SEQ ID NO:7) and T7 (5' TAATACGACTCACTATAGGG) (SEQ ID NO:8) primers flanking the cloning site were used to sequence both ends. Plasmid DNA (0.6 pmol) was used with 10.0 pmol of each primer for the dye terminator reaction. The SP6 end corresponded to the *in silico* derived EST sequence (identical to EST1). The T7 end did not have homologies with VR1 nor did it possess a long open reading frame or a polyadenylation motif. The size of the insert was determined by enzyme digestion of the DNA with the endonucleases NotI and EcoRI and calculated to be approximately 3kb.

Plasmid DNA (50ng) was used to amplify the insert by Polymerase Chain Reaction (PCR) with T7 and SP6 as primers. The PCR conditions included an initial hot-start at 94°C for 2 minutes, followed by 35 cycles at 94°C for 45 seconds, 50°C for 45 seconds and 72°C for 1 minute and terminated by 5 minutes at 72°C. The resulting PCR amplicon was separated on a 1.2% agarose gel and shown to be of ~3kb in size.

To fully sequence the PCR product the nuclease-Bal-31 technique was used where both strands of duplex DNA are degraded from both ends (23). After ethanol precipitation of the PCR product, the pellet was re-suspended in 30ml of 1X Bal-31 buffer (add buffer composition). A time-course digest with 2 units of Bal-31 enzyme (Roche Molecular Biochemicals) was carried out with 12 time points taken over 90 minutes (30 seconds, 1, 2, 3, 5, 7, 10, 15, 25, 45, 75 and 90 minutes). Three pools were made respectively from digests 1 to 4, 5 to 8 and 9

to 12. Each pool was blunt-ended and sub-cloned into the pCR-Script SK (+) plasmid from Stratagene at the SrfI site. After transformation, 16 colonies from each pool were screened by PCR with the flanking Reverse (5' GGAAACAGCTATGACCATG) (SEQ ID NO:9) and M13-20 (5' GTAAAACGACGGCCAGT) (SEQ ID NO:10) primers. The amplicons of 6 positive colonies per pool were subjected to direct sequencing (24) using the T3 (5' AATTAACCCTCACTAAAGGG) (SEQ ID NO:11) and T7 primers. The DNA sequences obtained were assembled using the GCG package, translated and aligned against the rat VR1 gene using the Blast tools. After analysis, the 3079bp amplicon was shown to have 2 introns of 603bp and 1221bp. The latter intron was located at the 3'end of the PCR product. The coding sequence covered 1255 bp and was separated by the former intron. Therefore the clone EST1 was likely to be a partially spliced and incomplete cDNA.

The clone belonging to cluster 1b (EST3) and derived from a kidney cDNA library was ordered and sequenced using the Bal-31 technique. After assembly of the sequences using the GCG package an identical overlap was identified with the DNA sequence of the cluster hVRc. Moreover a 3'end with a polyadenlyation signal and tail was identified. The complete sequence of the combined hVRb Bal-31 derived sequence and hVRc was 2063 bp (1020 bp of coding and 1043 bp of 3' untranslated sequence).

Reference Example B3: Amplification of the middle section of hVR1 using the Polymerase Chain Reaction

We formulated the hypothesis that both sequences (hVRa and hVRb/c) were part of a common transcript. If the human and rat VR1 were going to be similar, the 2 contigs should be separated by a gap of approximately 275bp. Primers were designed on both sides of the gap to amplify mRNA from brain tissues in order to clone the gap. A smear was obtained with the sense primer (5' TCTACTTCGGTGAACTGCCC) (SEQ ID NO:12) and antisense (5' ACGGCAGGGAGTCATTCTTC) (SEQ ID NO:13). For specificity 50ng of the PCR product were amplified with the nested sense (5' CTGCAGAACTCCTGGCAGA) (SEQ ID NO:14) and antisense (5' GTCACCACCGCTGTGGAAAA) (SEQ ID NO:15) primers. The 900bp nested amplicon was sequenced and shown to be identical to hVRa at one end and

hVRb/c at the other end. The middle part of the PCR product was homologous to the rat VR1 sequence. This region corresponded to 91 amino acids. When the sequences of hVRa, hVRb/hVRc and the internal amplicon are combined the total length of the Open Reading Frame (ORF) is 824 amino acids followed by a 3' untranslated sequence of 1043 bp. The human amino acid sequence is 87% identical to the rat sequence over that part of the coding region. This sequence was termed hVR1 because of its high degree of identity with the rat VR1 sequence.

Reference Example B4: Isolation of the 5' Terminus of hVR1 by PAC isolation

Since no start codon was identified at the 5' end an additional strategy was designed to identify the full-length sequence. Two primers, sense (5' TCCTCTGGCTTCCAACCCGTT) (SEQ ID NO:16) and antisense (5' GAACTGGGCAGAAAGTGCCT) (SEQ ID NO:17) were designed to amplify a 150bp product from the first intron mentioned in reference example B2. A P1 Artificial Chromosome (PAC) genomic clone (25) was isolated by PCR screening of a PAC library (Genome Systems, St Louis, Missouri). PAC DNA was recovered by using standard plasmid isolation protocol (26). An anti-sense primer was designed (5' CTGGAGTTAGGGTCTCCATCC) (SEQ ID NO:18) to sequence the genomic clone towards the potential 5' end of the gene. An open reading frame with a starting codon was identified. The gene structure was confirmed by using the GenScan software (27). The complete gene has a nucleotide sequence of 2517bp (figure 2) and encoded a 839 amino acid protein (Figures 3 and 4). The gene was named hVR1. Multiple alignment of the amino acid sequence of hVR1 and rat VR1 shows a remarkable degree of identity and similarities between both sequences (figure 5). The rVR1 and hVR1 amino acid sequences are 86% identical. Moreover after protein analysis 6 trans-membrane domains and 3 ankyrin binding domains were identified in hVR1 as in the rat VR1 gene.

<u>Example 1: Full-length Amplification of hVR1 from human DRG and assembly into cloning vectors</u>

HVR1 was PCR amplified in three sections from human DRG template. The 5' fragment was amplified using a sense primer encoding a NotI site and a strong

Kozak motif followed by gene specific sequence (5' GTCATAGCGGCCGCGCCGCCACCATGAAGAAATGGAGCAGCAC) (SEQ ID NO:19) and an antisense primer (5' AGGCCCACTCGGTGAACTTC) (SEQ ID NO:20). The thermo-cycling conditions used for this amplification included a hot start at 94°C for 4 mins, followed by 35 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 1 min. A final extension step of 72°C for 5 min completed the reaction. The resulting PCR products were separated on a 2% agarose gel and cloned into pCR®II-TOPO according to the manufacturers instructions supplied with the TOPO™ TA Cloning® kit (Invitrogen). The middle section of hVR1 was PCR amplified using the sense primer: 5' GACGAGCATGTACAATGAGA (SEQ ID NO:21) and antisense primer: 5' GTCACCACCGCTGTGGAAAA (SEQ ID NO:22). The cycling conditions included a hot start at 94°C for 4 mins, followed by 35 cycles of 1 min at 94°C, 56°C and 72°C. A final extension step of 72°C for 5 min completed the reaction. A band of approximately 870 bp was excised from a 2 % agarose gel and cloned as detailed by the TOPO™ TA Cloning® kit into the vector pCR2.1®-TOPO. Finally the 3' end was PCR amplified with the sense primer: 5' TGTGGACAGCTACAGTGAGA (SEQ ID NO:23) and the antisense primer: 5'TGCACTGAATTCGAGCACTGGTGTTCCCTCAG (SEQ ID NO:24) which encoded an EcoRI site for cloning. The PCR conditions included a 90 sec hot start at 94°C followed by 35 cycles of 94°C for 50 sec, 50°C for 50 sec and 72°C for 50 sec. The cycling was completed with a 72°C step for 5 min. PCR products were separated on a 2% agarose gel and cloned into the vector pCR2.1®-TOPO.

Resulting clones for each of the three hVR1-fragments were taken for sequence analysis and separate clones coding a consensus sequence were used in the full length assembly of the gene. The NotI/DraIII (New England Biolabs) digested 5' end fragment ligated together with the middle DraIII/EcoRI fragment into a NotI/EcoRI restricted pBluescript SK (+) vector (Stratagene). Finally, the remaining 3' fragment was introduced into the resulting construct via MscI and EcoRI restriction sites, a map of the resulting construct is displayed in figure 6A.

Several clones were selected for sequence analysis to confirm that constructs still encoded the hVR1 consensus sequence. These were then digested with NotI/EcoRI and ligated into the mammalian expression vector pCIN5-new (a modified version of pCIN1 (32) having an IVS deletion as well as a 36 bp

deletion repositioning the start codon of neomycin phosphotransferase immediately after the upstream EMVC IRES) as illustrated in figure 6B.

Example 2: Chromosomal Localisation

The primers used to isolate the PAC clone (reference example B4) were selected for PCR on the G3 radiation hybrid panel from Stanford commercially available from Research Genetics (Huntsville, Alabama). The positive lanes and negative patterns were analysed using the public web server at Stanford University (http://www-sghc.stanford.edu). After analysis the hVR1 gene appears to be located on human chromosome 17 around marker SHGC-36073 (lod score=9.55).

Example 3: mRNA Distribution

The tissue distribution of hVR1 was established by slot-blot hybridisation. RNA was transferred onto a sheet of GeneScreen hybridisation transfer membrane (DUPONT) sandwiched in a slot blotter by suction via a vacuum pump. Once the membrane was rinsed in 2x SSC (3M sodium chloride and 0.3M sodium citrate pH7) for 2 min it was exposed to UV using an Ultraviolet crosslinker (Amersham Life Science) for 1min at 15000uW/cm^2 thus enabling cross-linkage of the RNA onto the membrane. The amounts of RNA on the blot are unknown. The probe was obtained by PCR amplification of a 260 bp product of the coding region of hVR1 with the following two primers: 5' TGTGGACAGCTACAGTGAGA (SEQ ID NO:25) and 5' GTGGAAAACCCGAACAAGA (SEQ ID NO:26). Membranes were hybridised for 4 hr shaking at 60°C in a 10% dextran sulphate, 1% SDS (sodium dodecyl sulphate) and 1M NaCl solution. The probe was labelled with [α32P]dCTP (Amersham) using the Rediprime™DNA labelling system (Amersham), so as to obtain approximately 500,000cpm of the labelled probe per ml of prehybridisation solution. Briefly 100ng of probe was boiled for 3 minutes (denaturization) and then cooled on ice for 2 minutes in a total volume of 45µl. This was added to the labelling tube from the kit together with 3µl of 32P dCTP followed by an incubation at 37°C for 30 minutes. 400µl of Herring Sperm DNA (Sigma) at a concentration of 8µg/ml was added to the labelled probe and heated at 99°C for 3 minutes followed by rapid cooling on ice. The labelled probe was added and mixed well in pre-hybridisation solution. The membranes were hybridised overnight at 55°C.

The membranes were then washed, first at room temperature in 2xSSC and 1% SDS for 5 minutes, followed by 2x SSC and 1% SDS for 30 min at 50°C. If necessary further washes with 1x SSC and 0.5% SDS or 0.1xSSC and 0.1% for 30 mins at the same temperature were carried out. The membranes were then exposed to Scientific Imaging Film AR (Kodak) using intensifying screens at –70°C overnight and the film developed.

The results are shown on figure 7. Strong signals were observed with the positive controls (slots 4B and 5B). Signals are detected on the human DRG slots (1A and 1B). No signals were detected with the water control (slot 3B). Three multi-tissue northern blots (Clontech) with a wide range of tissues have also been hybridised with the same probe, however no signals were detected. RT-PCR was performed on various tissues with the primer combination used to amplify the probe. A strong band was detected in DRG RNA. Taken together these hybridisations suggest that hVR1 is specifically expressed in neuronal tissue and DRG in particular.

Example 4: Design and production of Anti-hVR1 Antibody

The peptides CHIFTTRSRTRLFGKGDSEEASC (SEQ ID NO:27) (peptide68) and CGSLKPEDAEVFKDSMVPGEK (SEQ ID NO:28) (peptide69) were synthesised by standard solid phase techniques and purified by gel filtration chromatography. These peptides were conjugated via their Cys residues to the carrier protein, Tuberculin PPD (purified protein derivative) using sulpho-SMCC (sulfosuccinimidyl 4-[N-maleimidomethyl]-cyclohexan-1-carboxylate). Rabbits, previously sensitised to Bacillus Calmette Guerin (BCG), were inoculated with the resulting conjugates emulsified in incomplete Freund's adjuvant at approx monthly intervals. Serum was prepared from blood samples taken 7 days after each immunisation. The specific antibody response was followed by indirect enzyme-linked immunosorbent assay (ELISA) using free peptide as antigen. Immunoglobulins were purified from high titre sera using immobilsed peptide affinity columns (sulpholink Pierce). Rabbits designated M143, 144 and 145 received peptide68 conjugate, rabbits M146, 147 and 148, peptide69 conjugate.

The antibodies have been validated by specific staining of the recombinant protein expressed in HEK293 cells. Whole cell lysates were prepared in Sample

peak heights are reduced in cells pre-incubated in CPZ. The same FLIPR assay may be used to monitor the response of human VR1 on exposure to agonists and antagonists.

Example 8: Example of a screen using human VR1.

FLIPR assay technology may be utilised to screen for hVR1 modulators according to the procedure described in figure 15. Human VR1 may be gated with protons, capsaicin or heat.

Reference Example C: Identification and partial characterisation of additional human vanilloid receptors (referenence examples C1-C3):

Reference Example C1: Identification and characterisation of a novel vanilloid–like receptor, hVR3

ESTs belonging to the remaining clusters were characterised by *in silico* cloning (reference example A). The following clones were used during this process: -EST6/EST7 (hVRd), -EST8. (hVRe), - EST9/EST10. (hVRf). These EST clusters have been aligned with rat VR1 in figure 16, note that this diagram is not to scale.

Reference Example C2: Sequencing of clones

Further sequencing, as detailed in reference example B2, and *in silico* cloning, enabled clusters hVRd, hVRe and hVRf to collapse forming a single contig of 583 amino acids. This sequence was named hVR3 and has 49 % identity with the rat VR1 sequence. It was unlikely that this single contig was a full-length vanilloid receptor transcript as no obvious starting codon was present and it was shorter than the rat VR1 transcript.

Reference Example C3: Identification of the 5' terminus of hVR3

Two primers (sense primer 5' ATGGCCACCAGCAGGGTTAC (SEQ ID NO:29) and antisense primer 5' TCTGCCAGGTTCCAGCTG) (SEQ ID NO:30) designed to PCR amplify an amplicon stretching the 3' end of hVR3 and its 3'utr were used to isolate a genomic PAC clone (Genome Systems. St Louis, Missouri). The hVR3 specific PAC clone was then used as template to generate a library. This was achieved by sonicating 6μg of Qiagen purified PAC construct, size selecting fragmented DNA of 500-

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2000bp. These resulting fragments were then blunt ended and cloned into the vector pCR®-Blunt as detailed in the manufacturers protocol supplied with the Zero Blunt™ PCR cloning kit (Invitrogen). Clones were then sequenced (reference example B2) to identify the complete 5' end of the hVR3 transcript. The full-length nucleotide sequence of the hVR3 gene is displayed in figure 17. Figure 18 illustrates both nucleotide and encoded amino acid sequence of the human VR1 and figure 19 depicts the amino acid sequence of the hVR3 gene with shaded regions denoting predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed).

Example 9: Full-length Amplification of hVR3 from human kidney template

Human kidney was used as a source of template for the PCR amplification of hVR3. Primers used for amplification were designed to isolate the gene in three fragments. Primers designed to isolate the 5' end included a sense primer encoding a NotI site and a strong Kozak motif followed by gene specific sequence (5' GTCATAGCGGCCGCGCCACCATGCCCAGGGTAGTTGGAC (SEQ ID NO: 31) and antisense primer (5' CACCTCTTGTTGTCACTGGA) (SEQ ID NO:32). The PCR conditions used were a hot start at 94°C for 4 mins, followed by 35 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min and finally one cycle at 72°C for 5 min. The resulting PCR products were separated on a 2% agarose gel and cloned into pCR®II-TOPO according to the manufacturers instructions supplied with the TOPO™ TA Cloning® kit (Invitrogen). The middle fragment was PCR generated using sense and antisense primers 5' CAAATCTGCGCATGAAGTTCCAG (SEQ ID NO:33) and 5' GCCACGAGAAGTTCCACGTAGTG (SEQ ID NO:34) respectively in the presence of 5% DMSO. PCR thermo-cycling required 35 cycles of 1 min at 94°C, 58°C and 72°C for successful amplification of the fragment which was then excised from a 2% agarose gel for cloning into the pCRII®-TOPO vector. Finally the 3' fragment was amplified with a sense primer 5' GCTGCTCCCATTCTTGCTGA (SEQ ID NO:35) and an antisense primer 5' TGCACTCTCGAGAAATGAGTGGGCAGAGAAGC (SEQ ID NO:36) encoding a XhoI restriction site. This fragment was successfully amplified using a hot start at 94°C for 4 min followed by 35 cycles of 94°C for 50 sec, 48°C for 50 sec and 72°C for 2 min. The cycling was completed with a 72°C step for 5 min. The amplified fragment was excised from a 2% agarose gel and clone into the pCRII®-TOPO vector.

Resulting clones for each of the three PCR generated hVR3-fragments were taken for sequence analysis and separate clones coding a consensus sequence were used in the full-length assembly of the gene. The DraIII restriction site of the pBluescript SK (+) vector (Stratagene) was firstly abolished by digestion with DraIII followed by a blunt ending step using T_4 DNA polymerase (New England Biolabs). This modified vector was then restricted to enable the ligation of both a NotI/NcoI 5' fragment and NcoI/ EcoRI middle fragment. Finally, the remaining 3' fragment was introduced into the resulting construct via DraIII and XhoI sites (figure 20A).

Several clones were selected for sequence analysis to confirm that the constructs still encoded the hVR3 consensus sequence. These were then digested with NotI/XhoI and ligated into the mammalian expression vector pCDNA3.1 (+) (Invitrogen) as seen in figure 20B. The resulting hVR3 consensus sequence is shown in the multiple alignment along with the full-length sequence of hVR1 and the published hVRL-1 in figure 21.

Example 10: Chromosomal localisation

The 3' terminus, including the 3' UTR sequence of hVR3 was used to design two primers to amplify a product of 360 bp: sense primer 5' ATGGCCACCAGCAGGGTTAC (SEQ ID NO:37) and antisense primer 5' TCTGCCAGGTTCCAGCTG (SEQ ID NO:38). The G3 radiation hybrid panel from Stanford University (Research Genetics, Huntsville, Alabama) was screened by PCR. The positive and negative lanes were analysed using the public web server at Stanford University (http://www-sghc.stanford.edu). After analysis the hVR3 gene appears to be located on human chromosome 12 around markers D12S177E (lod score=15) and D12S1893 (lod score=14).

Example 11: mRNA distribution

The following primers (5' ACAAGAAGGCGGACATGCGG (SEQ ID NO:39) and 5' ATCTCGTGGCGGTTCTCAAT) (SEQ ID NO:40) were used to obtain a PCR product from the coding region of hVR3. This amplicon was used as a probe on multi-tissue northern blots, the protocol of which is detailed in example 3, to determine the tissue distribution of the gene (figures 22A, 22B and 22C). A transcript of approximately 3.8 kb was detected in the following tissues (the intensities of the

FIG. 2

hVR1 SEQUENCE INCLUDING THE 5'UTR (nt -773 TO nt 0), CODING REGION (nt 1 TO 2517) AND 3'UTR (nt 2518 TO nt 3560) (SEQ ID NO:1)

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-773  ccccagccacacacacacacgcacacacatacacacacacacacaggcttaaccattca  -714
-713  aaggccagaagcttgacagatgttgattcataaaatgcaaaagccaaaatccaaaatct  -654
-653  tgtataagctcagtggctgtggcagcgaggttgaagagcaaaggcaggccgggcacctgg  -594
-593  ctgatgatgtgtggacccgttgcacagcagggcccgcagtgcggtgtgggtgtgggtggg  -534
-533  ccagtctctgccgctcaccctattccagggacacagtctgcttggctcttctggactgag  -474
-473  ccatcctcatcaccgagatcctccctgaattcagcccacgacagccaccccggccgtttt  -414
-413  ccttgttctgtgtgggaagggaggcagcgcggtggttatcaacctcaccctgcagaggag  -354
-353  gcacctgaggcccagagacgaggagggatgggtctaacccagaaccacagatggctctga  -294
-293  gccgggggcctgtccaccctcccaggccgacgtcagtggccgcaggactgcctgggccct  -234
-233  gctaggcctgctcacctctgaggcctctggggtgagaggttcagtcctggaaacacttca  -174
-173  gttctagggggctgggggcagcagcaagttggagttttggggtaccctgcttcacagggc  -114
-113  ccttggcaaggagggcaggtggggtctaaggacaagcagtccttactttgggagtcaacc  -54
 -53  ccggcgtggtggctgctgcaggttgcacactgggccacagaggatccagcaaggATGAAG  6
   7  AAATGGAGCAGCACAGACTTGGGGGCAGCTGCGGACCCACTCCAAAAGGACACCTGCCCA  66
  67  GACCCCCTGGATGGAGACCCTAACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCTCCACG  126
 127  GCCAAGAGCCGCACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTTCCCGGTGGAT  186
 187  TGCCCTCACGAGGAAGGTGAGCTGGACTCCTGCCCGACCATCACAGTCAGCCCTGTTATC  246
 247  ACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTGCTGTCCCAGGACTCTGTC  306
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FIG. 3

NUCLEOTIDE AND AMINO ACID SEQUENCE OF hVR1 INCLUDING (SEQ ID NO:1)
THE 5'UTR (nt -773 TO nt 0), CODING REGION (nt TO 2517) AND
3'UTR (nt 2518 TO nt 3560) (SEQ ID NO:2)

```
-773  cccccagccacacacacacacgcacacacatacacacacacacacaggcttaaccattca  -714

-713  aaggccagaagcttgacagatgttgattcataaaatgcaaaagccaaaatccaaaatct  -654

-653  tgtataagctcagtggctgtggcagcgaggttgaagagcaaaggcaggccgggcacctgg  -594

-593  ctgatgatgtgtggacccgttgcacagcagggcccgcagtgcggtgtgggtgtgggtggg  -534

-533  ccagtctctgccgctcaccctattccagggacacagtctgcttggctcttctggactgag  -474

-473  ccatcctcatcaccgagatcctccctgaattcagcccacgacagccaccccggccgtttt  -414

-413  ccttgttctgtgtgggaagggaggcagcgcggtggttatcaacctcaccctgcagaggag  -354

-353  gcacctgaggcccagagacgaggagggatgggtctaacccagaaccacagatggctctga  -294

-293  gccgggggcctgtccaccctcccaggccgacgtcagtggccgcaggactgcctgggccct  -234

-233  gctaggcctgctcacctctgaggcctctggggtgagaggttcagtcctggaaacacttca  -174

-173  gttctagggggctgggggcagcagcaagttggagttttggggtaccctgcttcacagggc  -114

-113  ccttggcaaggagggcaggtggggtctaaggacaagcagtccttactttgggagtcaacc  -54

 -53  ccggcgtggtggctgctgcaggttgcacactgggccacagaggatccagcaaggATGAAG  6
   1                                                         M  K    2

   7  AAATGGAGCAGCACAGACTTGGGGGCAGCTGCGGACCCACTCCAAAAGGACACCTGCCCA  66
   3  K  W  S  S  T  D  L  G  A  A  A  D  P  L  Q  K  D  T  C  P    22

  67  GACCCCCTGGATGGAGACCCTAACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCTCCACG  126
  23  D  P  L  D  G  D  P  N  S  R  P  P  P  A  K  P  Q  L  S  T    42

 127  GCCAAGAGCCGCACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTTCCCGGTGGAT  186
  43  A  K  S  R  T  R  L  F  G  K  G  D  S  E  E  A  F  P  V  D    62

 187  TGCCCTCACGAGGAAGGTGAGCTGGACTCCTGCCCGACCATCACAGTCAGCCCTGTTATC  246
  63  C  P  H  E  E  G  E  L  D  S  C  P  T  I  T  V  S  P  V  I    82

 247  ACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTGCTGTCCCAGGACTCTGTC  306
  83  T  I  Q  R  P  G  D  G  P  T  G  A  R  L  L  S  Q  D  S  V    102

 307  GCCGCCAGCACCGAGAAGACCCTCAGGCTCTATGATCGCAGGAGTATCTTTGAAGCCGTT  366
 103  A  A  S  T  E  K  T  L  R  L  Y  D  R  R  S  I  F  E  A  V    122

 367  GCTCAGAATAACTGCCAGGATCTGGAGAGCCTGCTGCTCTTCCTGCAGAAGAGCAAGAAG  426
 123  A  Q  N  N  C  Q  D  L  E  S  L  L  L  F  L  Q  K  S  K  K    142

 427  CACCTCACAGACAACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGCTGAAAGCC  486
 143  H  L  T  D  N  E  F  K  D  P  E  T  G  K  T  C  L  L  K  A    162

 487  ATGCTCAACCTGCACGACGGACAGAACACCACCATCCCCCTGCTCCTGGAGATCGCGCGG  546
```

REPLACEMENT PAGE

FIG. 4

AMINO ACID SEQUENCE OF hVR1 (SEQ ID NO:2)

```
  1 MKKWSSTDLG AAADPLQKDT CPDPLDGDPN SRPPPAKPQL STAKSRTRLF
 51 GKGDSEEAFP VDCPHEEGEL DSCPTITVSP VITIQRPGDG PTGARLLSQD
101 SVAASTEKTL RLYDRRSIFE AVAQNNCQDL ESLLLFLQKS KKHLTDNEFK
151 DPETGKTCLL KAMLNLHDGQ NTTIPLLLEI ARQTDSLKEL VNASYTDSYY
201 KGQTALHIAI ERRNMALVTL LVENGADVQA AHGDFFKKT KGRPGFYFGE
251 LPLSLAACTN QLGIVKFLLQ NSWQTADISA RDSVGNTVLH ALVEVADNTA
301 DNTKFVTSMY NEILILGAKL HPTLKLEELT NKKGMTPLAL AAGTGKIGVL
351 AYILQREIQE PECRHLSRKF TEWAYGPVHS SLYDLSCIDT CEKNSVLEVI
401 AYSSSETPNR HDMLLVEPLN RLLQDKWDRF VKRIFYFNFL VYCLYMIIFT
451 MAAYYRPVDG LPPFKMEKIG DYFRVTGEIL SVLGGVYFFF RGIQYFLQRR
501 PSMKTLFVDS YSEMLFFLQS LFMLATVVLY FSHLKEYVAS MVFSLALGWT
551 NMLYYTRGFQ QMGIYAVMIE KMILRDLCRF MFVYIVFLFG FSTAVVTLIE
601 DGKNDSLPSE STSHRWRGPA CRPPDSSYNS LYSTCLELFK FTIGMGDLEF
651 TENYDFKAVF IILLLAYVIL TYILLLNMLI ALMGETVNKI AQESKNIWKL
701 QRAITILDTE KSFLKCMRKA FRSGKLLQVG YTPDGKDDYR WCFRVDEVNW
751 TTWNTNVGII NEDPGNCXGV KRTLSFSLRS SRVSGRHWKN FALVPLLREA
801 SARDRQSAQP EEVYLRQFSG SLKPEDAEVF KSPAASGEK*
```

Key

T/S predicted phosphorylation sites

Transmembrane domains

Ankyrin binding domains

REPLACEMENT PAGE

FIG. 5

COMPARISON OF THE AMINO ACID SEQUENCE OF THE RAT (VR1) (SEQ ID NO:3) AND HUMAN (hVR1) VANILLOID PROTEINS. (SEQ ID NO:2)

```
              10        20        30        40        50
VR1    MEQRASLDSEESESPPQENSCLDPPDRDPNCKPPPVKPHIFTTRSRTRLF
hVR1   MKKWSSTDLGAAADPLQKDTCPDPLDGDPNSRPPPAKPQLSTAKSRTRLF
              60        70        80        90       100
VR1    GKGDSEEASPLDCPYEEGGLASCPIITVSSVLTIQRPGDGPASVRPSSQD
hVR1   GKGDSEEAFPVDCPHEEGELDSCPTITVSPVITIQRPGDGPTGARLLSQD
             110       120       130       140       150
VR1    SVSAG.EKPPRLYDRRSIFDAVAQSNCQELESLLPFLQRSKKRLTDSEFK
hVR1   SVAASTEKTLRLYDRRSIFEAVAQNNCQDLESLLLFLQKSKKHLTDNEFK
             160       170       180       190       200
VR1    DPETGKTCLLKAMLNLHNGQNDTIALLLDVARKTDSLKQFVNASYTDSYY
hVR1   DPETGKTCLLKAMLNLHDGQNTTIPLLLEIARQTDSLKELVNASYTDSYY
             210       220       230       240       250
VR1    KGQTALHIAIERRNMTLVTLLVENGADVQAAANGDFFKKTKGRPGFYFGE
hVR1   KGQTALHIAIERRNMALVTLLVENGADVQAAAHGDFFKKTKGRPGFYFGE
             260       270       280       290       300
VR1    LPLSLAACTNQLAIVKFLLQNSWQPADISARDSVGNTVLHALVEVADNTV
hVR1   LPLSLAACTNQLGIVKFLLQNSWQTADISARDSVGNTVLHALVEVADNTV
             310       320       330       340       350
VR1    DNTKFVTSMYNEILILGAKLHPTLKLEEITNRKGLTPLALAASSGKIGVL
hVR1   DNTKFVTSMYNEILILGAKLHPTLKLEELTNKKGMTPLALAAGTGKIGVL
             360       370       380       390       400
VR1    AYILQREIHEPECRHLSRKFTEWAYGPVHSSLYDLSCIDTCEKNSVLEVI
hVR1   AYILQREIQEPECRHLSRKFTEWAYGPVHSSLYDLSCIDTCEKNSVLEVI
             410       420       430       440       450
VR1    AYSSSETPNRHDMLLVEPLNRLLQDKWDRFVKRIFYFNFFVYCLYMIIFT
hVR1   AYSSSETPNRHDMLLVEPLNRLLQDKWDRFVKRIFYFNFLVYCLYMIIFT
             460       470       480       490       500
VR1    AAAYYRPVEGLPPYKLKWTVGDYFRVTGEILSVSGGVYFFFRGIQYFLQR
hVR1   MAAYYRPVDGLPPFKMEK.IGDYFRVTGEILSVLGGVYFFFRGIQYFLQR
             510       520       530       540       550
VR1    RPSLKSLFVDSYSEILFFVQSLFMLVSVVLYFSQRKEYVASMVFSLAMGW
hVR1   RPSMKTLFVDSYSEMLFFLQSLFMLATVVLYFSHLKEYVASMVFSLALGW
             560       570       580       590       600
VR1    TNMLYYTRGFQQMGIYAVMIEKMILRDLCRFMFVYLVFLFGFSTAVVTLI
hVR1   TNMLYYTRGFQQMGIYAVMIEKMILRDLCRFMFVYIVFLFGFSTAVVTLI
             610       620       630       640       650
VR1    EDGKNNSLPMESTPHKCRGSACK.PGNSYNSLYSTCLELFKFTIGMGDLE
hVR1   EDGKNDSLPSESTSHRWRGPACRPPDSSYNSLYSTCLELFKFTIGMGDLE
             660       670       680       690       700
VR1    FTENYDFKAVFIILLLAYVILTYILLLNMLIALMGETVNKIAQESKNIWK
hVR1   FTENYDFKAVFIILLLAYVILTYILLLNMLIALMGETVNKIAQESKNIWK
             710       720       730       740       750
VR1    LQRAITILDTEKSFLKCMRKAFRSGKLLQVGFTPDGKDDYRWCFRVDEVN
hVR1   LQRAITILDTEKSFLKCMRKAFRSGKLLQVGYTPDGKDDYRWCFRVDEVN
             760       770       780       790       800
VR1    WTTWNTNVGIINEDPGNCEGVKRTLSFSLRSGRVSGRNWKNFALVPLLRD
hVR1   WTTWNTNVGIINEDPGNCEGVKRTLSFSLRSSRVSGRHWKNFALVPLLRE
             810       820       830
VR1    ASTRDRHATQQEEVQLKHYTGSLKPEDAEVFKDSMVPGEK
hVR1   ASARDRQSAQPEEVYLRQFSGSLKPEDAEVFKSPAASGEK
```

FIG. 17

hVR3 SEQUENCE INCLUDING 5' UTR (nt -686 TO nt 0) CODING REGION (nt1 TO nt 2889), 3'UTR (nt 2890 TO nt 3418) (SEQ ID NO:4)

```
-684  ttacgcgttaagaaatacccaagcttatgcatcaagcttggtaccgagctcggatccact  -625

-624  agtaccgccggccagtgtgctggaattcaaggtgaggagaggagcatggatcctgggagc  -565

-564  gagtgtgtgcaggccagggagggctttccagaggagcccagttgagctggaacaccagtg  -505

-504  gggaggagttgaccagcaaaggtgcagggagggatcagcactttgcactggggagcagag  -445

-444  tttgtgcactggggaagtcaactcaagtattggagcctcagtttcctgttctgtaaaatg  -385

-384  ggttcatcatgacagtgtttgatgaggaaaaggactgccggcctacacagcaagtccaca  -325

-324  tggattttctgagcccctcctgtgcctgaagcccacggttaatggttctgccttagcagg  -265

-264  tgcttaccacgtgccaggcactgcactgcactggccactggactgcatgttctgtccatg  -205

-204  aggcttggatatccccatcttacagatcaggaagctgaggctatgaaatgtcgacttgct  -145

-144  caatgtcatggaatgactaagtgtggagcctggatttgaacttggctctctggggctcca  -85

 -84  aagctggctttcttggtcagcagtagggtctgggatccaagtatggggtcccagcttgac  -25

 -24  cctgaagtccaccctctttcagctaATGCCCAGGGTAGTTGGACCTGGGGCCAATTTGTG  35

  36  TTTCCAGGTTCGTGAAAGAGGCTCCTGTTGCAGTTCCCGCCTGAGGCTGGCGGCCAACCA  95

  96  CATCTGGGAGTGGCCTCCCTGTGCCCCTGTCATTACAACGGTGGCTTTGAAGCAGCTGGC  155

 156  AGCACTGCTGCTTGTCCACGTGGGAGGGGGCTTCCTGGAGCCCCCGCCCCTGGCCGGGTT  215

 216  CTGCCTGACTCCCCTTTCATTCCCTTGCAGGCTGAGCAGTGCAGACGGGCCTGGGGCAGG  275

 276  CATGGCGGATTCCAGCGAAGGCCCCCGCGCGGGGCCCGGGAGGTGGCTGAGCTCCCCGG  335

 336  GGATGAGAGTGGCACCCCAGGTGGGGAGGCTTTTCCTCTCTCCTCCCTGGCCAATCTGTT  395
```

FIG. 18

NUCLEOTIDE AND AMINO ACID SEQUENCE OF hVR3 (SEQ ID NO:4) INCLUDING THE 5'UTR (nt -684 TO nt 0), CODING REGION (nt1 TO 2889) AND 3'UTR (nt 2890 TO nt 3418) (SEQ ID NO:5)

```
-684  ttacgcgttaagaaatacccaagcttatgcatcaagcttggtaccgagctcggatccact  -625

-624  agtaccgccggccagtgtgctggaattcaaggtgaggagaggagcatggatcctgggagc  -565

-564  gagtgtgtgcaggccagggagggctttccagaggagcccagttgagctggaacaccagtg  -505

-504  gggaggagttgaccagcaaaggtgcagggagggatcagcactttgcactggggagcagag  -445

-444  tttgtgcactggggaagtcaactcaagtattggagcctcagtttcctgttctgtaaaatg  -385

-384  ggttcatcatgacagtgtttgatgaggaaaggactgccggcctacacagcaagtccaca  -325

-324  tggattttctgagcccctcctgtgcctgaagcccacggttaatggttctgccttagcagg  -265

-264  tgcttaccacgtgccaggcactgcactgcactggccactggactgcatgttctgtccatg  -205

-204  aggcttggatatccccatcttacagatcaggaagctgaggctatgaaatgtcgacttgct  -145

-144  caatgtcatggaatgactaagtgtggagcctggatttgaacttggctctctggggctcca  -85

 -84  aagctggctttcttggtcagcagtagggtctgggatccaagtatggggtcccagcttgac  -25

 -24  cctgaagtccaccctctttcagctaATGCCCAGGGTAGTTGGACCTGGGGCCAATTTGTG  35
   1                           M  P  R  V  V  G  P  G  A  N  L  C   12

  36  TTTCCAGGTTCGTGAAAGAGGCTCCTGTTGCAGTTCCCGCCTGAGGCTGGCGGCCAACCA  95
  13   F  Q  V  R  E  R  G  S  C  C  S  S  R  L  R  L  A  A  N  H   32

  96  CATCTGGGAGTGGCCTCCCTGTGCCCCTGTCATTACAACGGTGGCTTTGAAGCAGCTGGC  155
  33   I  W  E  W  P  P  C  A  P  V  I  T  T  V  A  L  K  Q  L  A   52

 156  AGCACTGCTGCTTGTCCACGTGGGAGGGGGCTTCCTGGAGCCCCCGCCCCTGGCCGGGTT  215
  53   A  L  L  L  V  H  V  G  G  G  F  L  E  P  P  P  L  A  G  F   72

 216  CTGCCTGACTCCCCTTTCATTCCCTTGCAGGCTGAGCAGTGCAGACGGGCCTGGGGCAGG  275
  73   C  L  T  P  L  S  F  P  C  R  L  S  S  A  D  G  P  G  A  G   92

 276  CATGGCGGATTCCAGCGAAGGCCCCCGCGCGGGGCCCGGGGAGGTGGCTGAGCTCCCCGG  335
  93   M  A  D  S  S  E  G  P  R  A  G  P  G  E  V  A  E  L  P  G   112

 336  GGATGAGAGTGGCACCCCAGGTGGGGAGGCTTTTCCTCTCTCCTCCCTGGCCAATCTGTT  395
 113   D  E  S  G  T  P  G  G  E  A  F  P  L  S  S  L  A  N  L  F   132

 396  TGAGGGGGAGGATGGCTCCCTTTCGCCCTCACCGGCTGATGCCAGTCGCCCTGCTGGCCC  455
 133   E  G  E  D  G  S  L  S  P  S  P  A  D  A  S  R  P  A  G  P   152

 456  AGGCGATGGGCGACCAAATCTGCGCATGAAGTTCCAGGGCGCCTTCCGCAAGGGGGTGCC  515
 153   G  D  G  R  P  N  L  R  M  K  F  Q  G  A  F  R  K  G  V  P   172

 516  CAACCCCATCGATCTGCTGGAGTCCACCCTATATGAGTCCTCGGTGGTGCCTGGCCCAA  575
 173   N  P  I  D  L  L  E  S  T  L  Y  E  S  S  V  V  P  G  P  K   192
```

REPLACEMENT PAGE

FIG. 19

AMINO ACID SEQUENCE OF hVR3 (SEQ ID NO:5)

```
  1 MPRVVGPGAN LCFQVRERGS CCSSRLRLAA NHIWEWPPCA PVITTVALKQ
 51 LAALLLVHVG GGFLEPPPLA GFCLTPLSFP CRLSSADGPG AGMADSSEGP
101 RAGPGEVAEL PGDESGTPGG EAFPLSSLAN LFEGEDGSLS PSPADASRPA
151 GPGDGRPNLR MKFQGAFRKG VPNPIDLLES TLYESSVVPG PKKAPMDSLF
201 DYGTYRHHSS DNKRWRKKII EKQPQSPKAP APQPPPILKV FNRPILFDIV
251 SRGSTADLDG LLPFLLTHKK RLTDEEFREP STGKTCLPKA LLNLSNGRND
301 TIPVLLDIAE RTGNMREFIN SPFRDIYYRG QTALHIAIER RCKHYVELLV
351 AQGADVHAQA RGRFFQPKDE GGYFYFGELP LSLAACTNQP HIVNYLTENP
401 HKKADMRRQD SRGNTVLHAL VAIADNTREN TKFVTKMYDL LLLKCARLFP
451 DSNLEAVLNN DGLSPLMMAA KTGKIGIFQH IIRREVTDED TRHLSRKSKD
501 WAYGPVYSSL YDLSSLDTCG EEASVLEILV YNSKIENRHE MLAVEPINEL
551 LRDKWRKFGA VSFYINVVSY LCAMVIETLT AYYQPLEGTP PYPYRTTVDY
601 LRLAGEVITL FTGVLFFFTN IKDLFMKKCP GVNSLFIDGS FQLLYFIYSV
651 LVIVSAALYL AGIEAYLAMM VFALVLGWMN ALYFTRGLKL TGTYSIMIQK
701 ILFKDLFRFL LVYLLFMIGY ASALVSLLNP CANMKVCNED QTNCTVPTYP
751 SCRDSETFST FLLDLFKLTI GMGDLEMLSS TKYPVVFIIL LVTYIILTSV
801 LLLNMLIALM GETVGQVSKE SKHIWKLQWA TTILDIERSF PVFLRKAFRS
851 GEMVTVGKSS DGTPDRRWCF RVDEVNWSHW NQNLGIINED PGKNETYQYY
901 GFSHTVGRLR RDRWSSVVPR VVELNKNSNP DEVVVPLDSM GNPRCDGHQQ
951 GYPRKWRTDD APL
```

Key

Transmembrane domains

Ankyrin binding domains

REPLACEMENT PAGE

FIG. 21

A MULTIPLE COMPARISON OF THE AMINO ACID SEQUENCES OF THE RAT VR1 AND THE HUMAN VANILLOID RECEPTORS, hVR1, hVRL-1 AND hRV3

```
                    10        20        30        40        50
VR1     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~     (SEQ ID NO:3)
hVR1    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~     (SEQ ID NO:2)
hVRL-1  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~     (SEQ ID NO:6)
hVR3    MPRVVGPGANLCFQVRERGSCCSSRLRLAANHIWEWPPCAPVITTVALKQ     (SEQ ID NO:5)

                    60        70        80        90       100
VR1     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR1    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVRL-1  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR3    LAALLLVHVGGGFLEPPPLAGFCLTPLSFPCRLSSADGPGAGMADSSEGP

                   110       120       130       140       150
VR1     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~MEQRASLDSEESESPPQENSCL
hVR1    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~MKKWSSTDLGAAADPLQKDTCP
hVRL-1  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
hVR3    RAGPGEVAELPGDESGTPGGEAFPLSSLANLFEGEDGSLSPSPADASRPA

                   160       170       180       190       200
VR1     DPPDRDPNCKPPPVKPHIFTTRSRTRLFG....KGDSEEASPLDCPYEEG
hVR1    DPLDGDPNSRPPPAKPQLSTAKSRTRLFG....KGDSEEAFPVDCPHEEG
hVRL-1  ~~~~~~~~~~~~~~~~~~MTSPSSSPVFRLETLDGGQEDGSEADRGKLDF
hVR3    GPGDGRPNLRMKFQGAFRKGVPNP.....IDLLESTLYESSVVPGPKKAP

                   210       220       230       240       250
VR1     GLASCPIITVSSVLTIQRPGDGPASVRPSSQDSVSAG.EKP.PRLYDRRS
hVR1    ELDSCPTIITVSPVITIQRPGDGPTGARLLSQDSVAASTEKT.LRLYDRRS
hVRL-1  GSGLPPM..ESQFQGEDRKFAPQIRVNLNYRKGTGASQPDP.NR.FDRDR
hVR3    MDSLFDYGTYRHHSSDNKRWRKKIIEKQPQSPKAPAPQPPPILKVFNRPI

                   260       270       280       290       300
VR1     IFDAVAQSNCQELESLLPFLQRSKKRLTDSEFKDPETGKTCLLKAMLNLH
hVR1    IFEAVAQNNCQDLESLLLFLQKSKKHLTDNEFKDPETGKTCLLKAMLNLH
hVRL-1  LFNAVSRGVPEDLAGLPEYLSKTSKYLTDSEYTEGSTGKTCLMKAVLNLK
hVR3    LFDIVSRGSTADLDGLLPFLLTHKKRLTDEEFREPSTGKTCLPKALLNLS

                   310       320       330       340       350
VR1     NGQNDTIALLLDVARKTDSLKQFVNASYTDSYYKGQTALHIAIERRNMTL
hVR1    DGQNTTIPLLLEIARQTDSLKELVNASYTDSYYKGQTALHIAIERRNMAL
hVRL-1  DGVNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRSLQC
hVR3    NGRNDTIPVLLDIAERTGNMREFINSPFRDIYYRGQTALHIAIERRCKHY

                   360       370       380       390       400
VR1     VTLLVENGADVQAAANGDFFKKTKGRPGFYFGELPLSLAACTNQLAIVKF
hVR1    VTLLVENGADVQAAAHGDFFKKTKGRPGFYFGELPLSLAACTNQLGIVKF
hVRL-1  VKLLVENGANVHARACGRFFQKGQG.TCFYFGELPLSLAACTKQWDVVSY
hVR3    VELLVAQGADVHAQARGRFFQPKDEGGYFYFGELPLSLAACTNQPHIVNY

                   410       420       430       440       450
VR1     LLQNSWQPADISARDSVGNTVLHALVEVADNTVDNTKFVTSMYNEILILG
hVR1    LLQNSWQTADISARDSVGNTVLHALVEVADNTADNTKFVTSMYNEILMLG
hVRL-1  LLENPHQPASLQATDSQGNTVLHALVMISDNSAENIALVTSMYDGLLQAG
hVR3    LTENPHKKADMRRQDSRGNTVLHALVAIADNTRENTKFVTKMYDLLLLKC

                   460       470       480       490       500
VR1     AKLHPTLKLEEITNRKGLTPLALAASSGKIGVLAYILQREIHEPECRHLS
hVR1    AKLHPTLKLEELTNKKGMTPLALAAGTGKIGVLAYILQREIQEPECRHLS
hVRL-1  ARLCPTVQLEDIRNLQDLTPLKLAAKEGKIEIFRHILQREFS..GLSHLS
hVR3    ARLFPDSNLEAVLNNDGLSPLMMAAKTGKIGIFQHIIRREVTDEDTRHLS
```

REPLACEMENT PAGE